

QUANTITATIVE ANALYSIS OF HYDROPEROXYL RADICAL USING FLOW
INJECTION ANALYSIS WITH CHEMILUMINESCENCE DETECTION

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Quantitative Analysis of Hydroperoxyl Radical Using Flow Injection Analysis with Chemiluminescence Detection

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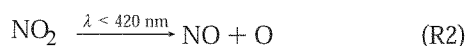
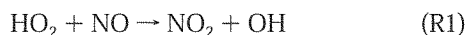
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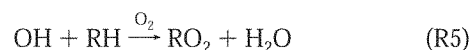
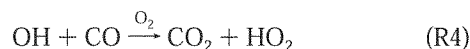
The hydroperoxyl radical (HO_2) is one of the most abundant free radicals in the atmosphere, where it participates in a series of photochemical reactions that determine the fate of natural and anthropogenic emissions. In addition, HO_2 is found in droplets and surface water as a result of photochemical formation and gas-phase scavenging. We describe a quantitative method for determining trace concentrations of HO_2 radicals that exploits the chemiluminescence produced upon reaction with a synthetic analogue of luciferin from the crustacean *Cypridina*. The technique is linear at least up to $1 \mu\text{M}$ $\text{HO}_2(\text{aq})$ and has a minimum detection limit of 0.1 nM . A unique feature of this analysis is a calibration method using stable aqueous HO_2 standards produced in submicromolar concentrations using ^{60}Co γ -radiolysis. The advantage of this method in comparison to others in consideration of field deployment is its simplicity, low cost, and minimal size and power requirements. One intended application of this technique is the measurement of atmospheric HO_2 radicals following collection into aqueous solution.

The hydroperoxyl radical (HO_2) is one of the most abundant free radicals in the atmosphere. HO_2 plays an important role in urban smog formation through its reaction with NO to yield NO_2 , which is photolyzed in the troposphere to generate O_3



Peroxy radicals in the atmosphere originate from reaction of the hydroxyl radical with organic compounds in the presence of oxygen. Reaction of OH with CO forms HO_2 directly (R4), while

analogous reactions with other organic compounds yield alkylperoxy radicals, RO_2 via R5



Except in the remote troposphere, the fate of RO_2 is dictated by its reaction with NO to produce NO_2 and alkoxy radicals. Some alkoxy radicals subsequently may react to produce additional HO_2 .¹

Atmospheric HO_2 is highly soluble² and will partition from air into an available aqueous phase, where it equilibrates rapidly with its conjugate base, superoxide anion, $\text{O}_2^- (\text{aq})$:



Photochemical formation is another source of these radicals in surface waters and droplets.³ HO_2 has a pK_a of 4.8, and the acid and base forms generally exhibit very different aqueous-phase reactivities.⁴ Hereinafter, we will refer to the equilibrium mixture of HO_2 and O_2^- in aqueous solution as $\text{HO}_2(\text{aq})$; this term refers to the proper apportionment of HO_2 and O_2^- at the pH in question.

The low atmospheric abundance and high reactivity of HO_2 radicals, combined with the lack of a strong unique spectral signature or quantitative standards, presents an analytical challenge. Nevertheless, the past decade has seen the emergence of several sensitive, fast, and highly specific methods for the detection of these species in the atmosphere. The peroxy radical

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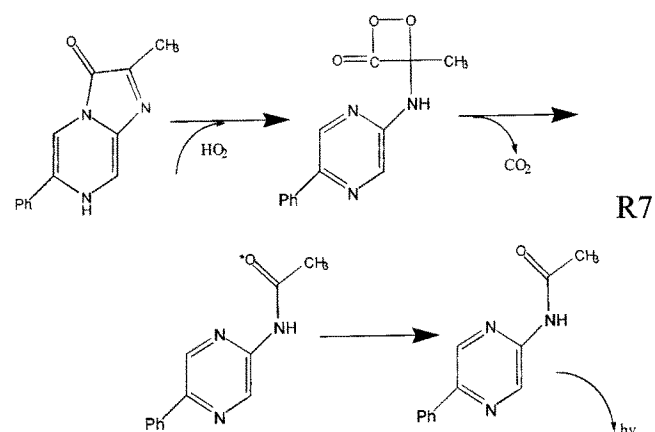
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chemical amplifier method, developed by Cantrell et al.,⁵ produces OH and NO₂ by the reaction of excess NO with HO₂ (R1). Excess CO is added to recycle OH back to HO₂ (R4), and the resulting chain-amplified NO₂ signal is measured by luminol chemiluminescence. This method has a 1 pptv detection limit but is subject to interference from atmospheric O₃, PAN, and NO₂. Determination of the chain length may introduce a significant error into the measurement.⁶ Mihelcic et al. have reported peroxy radical abundances using ESR spectroscopy after trapping the radicals in a D₂O matrix at 77 K.^{7,8} These investigators use a numerical fitting procedure that permits the determination of HO₂ in the presence of RO₂ with a detection limit of 5 pptv for a 30-min sampling interval. HO₂ also can be measured after reaction with excess NO, followed by detection of the resulting OH by laser-induced fluorescence (LIF) at 308 nm.^{9,10} LIF is highly specific for OH and exhibits a 0.03 pptv detection limit. A peroxy radical chemical ionization mass spectroscopy (PerCIMS) technique has been developed for the measurement of tropospheric HO₂ and RO₂.¹¹ For this measurement, excess NO is added to produce OH upon reaction with peroxy radicals. Hydroxyl radicals react rapidly with added isotopically labeled SO₂, and the resulting labeled sulfuric acid is detected by quadrupole mass spectrometry. PerCIMS has excellent sensitivity and may be able to discriminate between HO₂ and RO₂ by controlling the concentration of added NO and SO₂.

All of the methods described above have been deployed in recent field programs with varying degrees of success and are still very much under development. We describe here a continuous, fast, and specific HO₂ detector that is based on the chemiluminescent reaction between HO₂(aq) and a synthetic analogue of the luciferin from the crustacean *Cypridina*.

Biochemists have reported that certain proteins extracted from marine animals luminesce in the presence of activated granulocytes (e.g., refs 12 and 13). Emission of light from synthetic *Cypridina* luciferin analogue (CLA) in the superoxide-generating xanthine/xanthine oxidase system prompted early suggestions that HO₂(aq) was involved in the reaction.¹⁴ This was supported by the observation that the enzyme superoxide dismutase suppressed luminescence. The addition of mannitol, which scavenges hydroxyl radical, did not inhibit luminescence, nor did azide, which scavenges ¹O₂, nor did catalase, which destroys peroxide. CLA

luminescence is now widely used to demonstrate the putative involvement of HO₂(aq) in biological processes. A mechanism for reaction of CLA with HO₂ has been proposed (R7), and the pH



dependence of the reaction rate has been evaluated.¹⁵ The chemiluminescence spectrum depends on pH, peaking at 380 nm at pH 7.0 and 450 nm at pH 11.1.¹⁶ A growing literature now provides reaction rates of HO₂(aq) with various compounds measured by competition kinetics, using the CLA + HO₂(aq) rate constant as a reference.¹⁷ A number of CLA analogues have been synthesized; these vary in aqueous solubility, sensitivity to HO₂, stability versus pH, and background emission.¹⁸

We are investigating this family of chemiluminescence reagents for the quantitative analysis of HO₂. Previous investigations in biological systems have focused on the rate of generation of HO₂(aq), rather than the absolute concentration, because enzymatic radical generation systems produce variable quantities of HO₂(aq). Rather than using complex biological systems and their associated buffers, we produce known quantities of HO₂(aq) in aqueous solution for calibration standards using ⁶⁰Co γ-radiolysis. We take advantage of the elegant work of Bielski and Arudi,¹⁹ who showed that it is possible to produce stable solutions of O₂^{•-} at high pH. We have demonstrated that CLA and MCLA emit light upon reaction with radiolytically produced HO₂(aq) radicals and have examined potential interferences as a function of pH. We have incorporated the chemiluminescent HO₂(aq) + CLA chemistry into a flow injection analysis (FIA) apparatus similar to that used for determination of atmospheric hydroperoxides²⁰ and have shown it to be linear and sensitive to aqueous HO₂(aq). This paper describes the experimental apparatus and optimum conditions for the analysis of HO₂(aq) solutions.

EXPERIMENTAL SECTION

Reagents. All reagents used were the purest grade available, and all solutions were prepared using 18.2-MΩ water purified by

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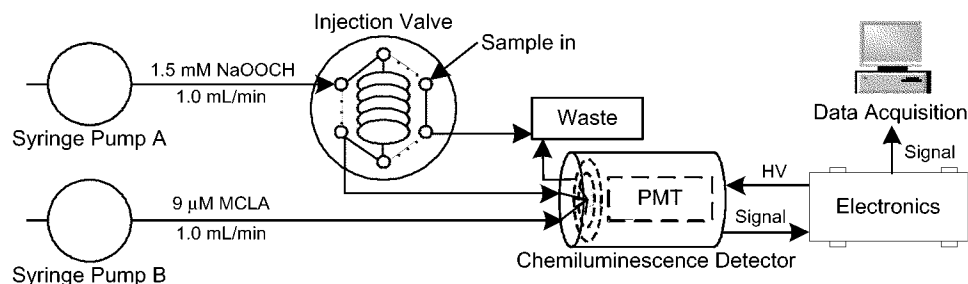
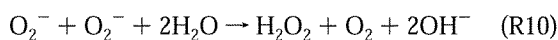
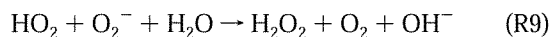
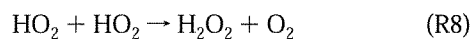


Figure 1. Schematic diagram of the FIA-based $\text{HO}_2(\text{aq})$ chemiluminescence detector.

a Millipore system (Billerica, MA). The *Cypridina* luciferin analogues 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (CLA) and 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA) were purchased from TCI America (Portland, OR) and used without purification. Sodium formate (Baker, Analyzed Reagent Grade) and sodium phosphate (GFS Chemicals) for radiolysis experiments were recrystallized twice, first from Millipore water containing 10^{-4} M EDTA to remove trace metal impurities and then from Millipore water alone. Sodium hydroxide (GFS Chemicals, 99.999%) and hydrochloric acid (Sigma Co., 99.999%) were used without further purification. Interference studies were conducted using H_2O_2 (3%, Mallinckrodt) and O_3 (Thermo Environmental model 165C ozone generator).

Aqueous Standards. O_2^- stock solutions were prepared by ^{60}Co γ -irradiation of air-saturated aqueous solutions containing 1.5 mM sodium formate and 5 μM EDTA, adjusted to pH 11 with NaOH. Under these conditions, the primary radicals H , OH , and e_{aq}^- are converted to an equilibrium mixture of O_2^- and HO_2 within milliseconds.²¹ In the absence of additives, O_2^- and HO_2 radicals decay by self-reactions R8–R10 with a pH-dependent rate coefficient (k_{obs}):



$$k_{\text{obs}} = \frac{k_8 + k_9(K_6/[\text{H}^+])}{(1 + K_6/[\text{H}^+])^2}$$

where $k_8 = 8.3 \times 10^5$, $k_9 = 9.7 \times 10^7$, and $k_{10} < 0.3 \text{ L mol}^{-1} \text{ s}^{-1}$.⁴ ^{60}Co source intensity, measured by ferrous sulfate dosimetry, was $2.77 \text{ rads min}^{-1}$, producing $0.290 \mu\text{M}$ $\text{HO}_2(\text{aq})$ radicals min^{-1} . At pH 11, a sample initially containing 1 μM O_2^- has a half-life of 2.8 h. We verify O_2^- concentration spectrophotometrically ($\epsilon_{240} = 2300 \text{ L mol}^{-1} \text{ cm}^{-1}$), immediately dilute the sample by a factor of 100, and store it at 0 $^\circ\text{C}$. Calibrations are conducted within 8 h using this diluted solution, which has an O_2^- half-life on the order of days.

Figure 1 is a schematic diagram of the apparatus. Two high-precision syringe pumps (model 260D, Isco Inc., Lincoln, NE)

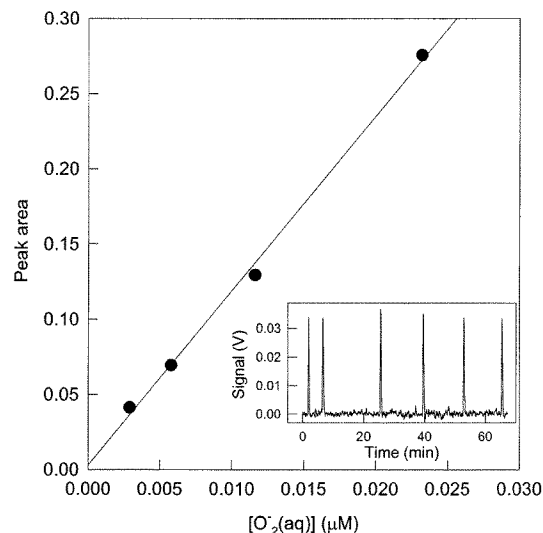


Figure 2. Calibration of the $\text{HO}_2(\text{aq})$ detector using radiolytic standards. Standards are air-saturated and contain 1.0 mM NaOOCH adjusted with NaOH to pH 11, irradiated by ^{60}Co for 1.0 min, and then diluted 1:100 into unirradiated formate solution. Inset shows stability of $\text{HO}_2(\text{aq})$ standards (peak area 0.732 ± 0.022). Standards contain 8.7 nM $\text{O}_2^-(\text{aq})$, 5.0 μM MCLA, sample volume 205 μL , and total flow rate 1.50 mL/min.

deliver a reagent stream and a carrier stream to a chemiluminescence detector (model 660, McPherson Inc., Chelmsford, MA) at 1.0 mL min^{-1} . The carrier stream consists of unirradiated formate solution adjusted to pH 11 with NaOH, and the reagent stream is a 9 μM MCLA solution in 1:100 (v/v) ethanol/ H_2O , adjusted to pH 2.3 with HCl. $\text{HO}_2(\text{aq})$ samples are injected into the carrier stream using a six-port injection valve (VICI, Houston, TX). The chemiluminescence detector has a 500- μL spiral reaction cell that permits reagents to mix directly in front of a Hamamatsu R-268 photomultiplier tube.

RESULTS AND DISCUSSION

Calibration. Figure 2 is a calibration curve using radiolytically produced standards, with an inset that shows that the standards are stable for more than 1 h, substantially longer than necessary for calibration. The pH of the mixed carrier and reagent stream is 3. At this pH, the half-life of $\text{HO}_2(\text{aq})$ with respect to reaction with MCLA is 1 s,¹⁵ while the self-reaction has a half-life of 30 s. Thus, there is no significant loss of $\text{HO}_2(\text{aq})$ to self-reaction during the calibration. The baseline-to-baseline width of the FIA signal is 50 s, which allows for an ~ 1 -min sampling frequency for

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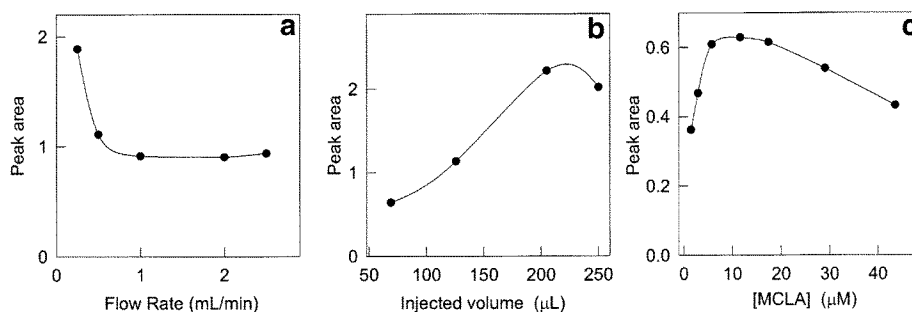


Figure 3. Determination of optimum conditions for detection of 2.9 nM HO₂(aq). Effect of (a) flow rate for 205 μL injected sample volume; (b) sample size for flow rate 0.5 mL/min, [MCLA] = 10 μM, [MCLA] = 10 μM; (c) MCLA concentration for 80 μL injected volume at 1.0 mL/min flow rate.

continuous measurements. The calibration is linear, with a detection limit of 0.12 nM, based on three times the baseline noise.

Optimization and Interferences. We varied chemical composition, flow rates, and sampling volume to determine the optimum reaction conditions. Signal area decreased with solution flow rates up to 1.0 mL min⁻¹ and became constant (Figure 3a). Peak area showed a maximum for an injected volume of 200 μL of HO₂(aq) (Figure 3b). An MCLA concentration of 12 μM produced the largest response (Figure 3c).

Samples of H₂O₂ and O₃ were tested to see if they generated a luminescence response from MCLA. Addition of 1 μM H₂O₂ generated a luminescence signal equivalent to 0.1 nM HO₂(aq), but we observed no response from an aqueous sample that had been saturated with a gaseous stream containing 150 ppbv O₃ in O₂.

It is worth noting that air oxidation of MCLA occurs at high pH, producing luminescence identical to that from HO₂,¹⁶ but the HO₂ standards must be kept at high pH to avoid the fast reaction R9. Thus, adjusting the pH to maintain the titer of both MCLA and HO₂ standards is critical.

DISCUSSION

We have demonstrated that MCLA can be used for the routine determination of HO₂(aq) in aqueous solution using FIA, with a detection limit of 1×10^{-10} M. Using ⁶⁰Co γ-radiolysis to generate HO₂(aq) samples, we have verified the fact that these radicals react with MCLA to generate luminescence. We have demonstrated that radiolytically produced HO₂(aq) samples can be used as calibration standards and have determined conditions for optimum detection. There are no published reports of the quantitative application of this technique to HO₂ radical detection (see, for example, a review of analytical applications of flow injection with chemiluminescence detection²²). For atmospheric applications, we plan to collect HO₂ using a high-pH stripping solution. Although the published Henry's law constant for HO₂ varies from 4×10^3 to 9×10^3 M atm⁻¹,²³ its large magnitude causes the collection efficiency to be rather insensitive to the exact value. For a liquid flow rate of 1 mL min⁻¹ and an air flow rate of 1 L min⁻¹, calculated collection efficiencies are 98.9 and 99.5%, respectively, for the high and low value of K_H . High-pH stripping

Table 1. Potentially Interfering Gaseous Oxidants

species	k_{11} (M/atm) ^a	atmos abund (ppb) ^b	collection efficiency ^c	expected concn (M)
H ₂ O ₂	7.4×10^4	5	99	4.0×10^{-7}
O ₃	1.1×10^{-2}	100	1.2×10^{-4}	9.7×10^{-10}
HO ₂	9×10^3	0.050	99	4.0×10^{-9}
OH	25	0.0005	0.22	8.7×10^{-12}
¹ O ₂	1.3×10^{-3d}	0.005	1.5×10^{-5}	6.1×10^{-15}

^a Reference 23. ^b Reference 28. ^c Based on 1 mL min⁻¹ liquid flow and 1 L min⁻¹ air flow in glass scrubbing coil. ^d Assumed to be the same as O₂.

solution will favor the dissociation of HO₂ to O₂⁻, increasing the effective Henry's law constant and further reducing the uncertainty in collection efficiency. These flow rates should result in a gas-phase detection limit of 1 pptv HO₂, adequate sensitivity for polluted areas where midday concentrations of HO₂ are expected to peak near 100 pptv.²⁴

Several investigators have published rate constants for the reaction between O₂⁻ + CLA, all using enzymatically produced O₂⁻. Two of these values, 2.12×10^5 M⁻¹ s⁻¹ at pH 7.8²⁵ and 6.7×10^4 M⁻¹ s⁻¹ at pH 8.2¹⁵ are of similar magnitude, while one, 1.08×10^8 M⁻¹ s⁻¹ at pH 7.0,²⁶ is very much higher. Reliable determination of the relevant pH-dependent rate constant is important, as the rate of the CLA + O₂⁻ reaction is now being used as a standard for competition kinetics for reactions of O₂⁻ with a variety of species.¹⁷ We are conducting a systematic pulse radiolysis study of the kinetics of reaction of the CLA family with HO₂(aq); results of this study will be reported elsewhere.

Because of the high sensitivity of the chemiluminescence method, it is critical to evaluate potential interferences from atmospheric oxidants that may be co-collected in the stripping coil. These species and their Henry's law solubilities are given in Table 1, together with ambient gas-phase abundances and the resulting aqueous concentrations expected in our apparatus under typical operating conditions. H₂O₂ is the most abundant water-soluble atmospheric oxidant and may be collected in the gas-aqueous scrubbing coil at concentrations in the micromolar range.²⁰ MCLA was reported to be unreactive toward H₂O₂ based

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on the inability of catalase enzyme to quench HO_2 -generated luminescence.¹⁴ As discussed earlier, we have confirmed that luminescence from reaction with H_2O_2 is minimal, but we are seeking experimental conditions that will reduce this interference. Although much less soluble than H_2O_2 , O_3 may be 100 times more abundant; absence of O_3 interference was therefore important to substantiate. Reactions with OH and $^1\text{O}_2$ are not expected to be a problem due to their extremely low aqueous-phase concentrations but will be explored in future work.

Loss of sample in inlet tubing is an important concern for HO_2 radicals due to their labile nature and high solubility in surface-adsorbed water. We plan to mitigate this problem with an "inletless" glass coil collection device that directs sampled air into an aqueous stripping solution through a 1-mm orifice.²⁷ This method prevents contact of gaseous HO_2 radicals with the wall.

In addition to kinetic studies of $\text{HO}_2(\text{aq})$ with MCLA and related chemiluminescence reagents, future work will focus on integrating the FIA detection system with a gas-phase sample collection apparatus. One important area of investigation will be

the extent to which the CLAs respond to organic peroxy radicals, e.g., CH_3O_2 and $\text{C}_2\text{H}_5\text{O}_2$, which are expected to be abundant in the atmosphere. These radicals are less water soluble than HO_2 and, in aqueous solution, are unlikely to form the dioxetane intermediate responsible for light emission, suggesting that they may not interfere. Studies of other potentially interfering species are ongoing.

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